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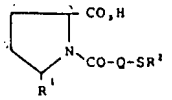
(54) Antihypertensive 5-Substituted 2-Pyrrolidinecarboxylic Acids and Salts Thereof

(57) Compounds of the formula (I) and salts thereof are useful as antihypertensive agents in pharmaceutical compositions:—



(I)

wherein R^1 is phenyl, hydroxyphenyl mercapto-lower alkyl, acylmercapto-lower alkyl, higher alkyl, higher alkenyl, cycloalkyl, aralkyl, aralkenyl, furyl, thienyl, imidazolyl, pyridyl, naphthyl, benzofuryl, benzothienyl, indolyl, substituted higher alkyl, substituted higher alkenyl, substituted cycloalkyl, substituted aralkyl, substituted aralkenyl, substituted phenyl, substituted furyl, substituted thienyl, substituted imidazolyl, substituted pyridyl, substituted naphthyl, substituted benzofuryl, substituted benzothienyl, or substituted indolyl wherein the substituent(s) is or are lower alkyl, hydroxy-lower alkyl, hydroxy, mercapto, lower alkoxy, lower alkylenedioxy, acyloxy, acylmercapto, halogen, nitro amino, lower alkylamino, acylamino or carboxy, except hydroxyphenyl;

R^2 is hydrogen or benzoyl; and

Q is a straight or branched chain alkylene with 1 to 3 carbon atoms, or a salt thereof.

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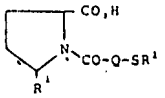
SPECIFICATION

Antihypertensive 5-Substituted 2-Pyrrolidinecarboxylic Acids and Salts Thereof

This invention relates to derivatives of 5-substituted 2-pyrrolidinecarboxylic acid and salts thereof which are useful as antihypertensive agents. These compounds are represented by the formula



(I)

wherein R^1 is phenyl, hydroxyphenyl, mercapto-lower alkyl, acylmercapto-lower alkyl, higher alkyl, higher alkenyl, cycloalkyl, aralkyl, aralkenyl, furyl, thienyl, imidazolyl, pyridyl, naphthyl, benzofuryl, benzothienyl, indolyl, substituted higher alkyl, substituted higher alkenyl, substituted cycloalkyl, substituted aralkyl, substituted aralkenyl, substituted phenyl, substituted furyl, substituted thienyl, substituted imidazolyl, substituted pyridyl, substituted naphthyl, substituted benzofuryl, substituted benzothienyl, or substituted indolyl wherein the substituent(s) is or are lower alkyl, hydroxy-lower alkyl, hydroxy, mercapto, lower alkoxy, lower alkylenedioxy, acyloxy, acylmercapto, halogen, nitro, amino, lower alkylamino, acylamino or carboxy, except hydroxyphenyl; R^2 is hydrogen or benzoyl; Q is a straight or branched alkylene with 1 to 3 carbon atoms (e.g. $—CH_2—$, $—CH_2CH_2—$, $—CH(CH_3)CH_2—$);

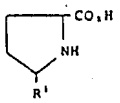
In This Specification:—

lower alkyl, alkenyl or alkylene means a saturated or unsaturated, straight or branched chain with 1 to 6 carbon atoms; higher alkyl or alkenyl means a saturated or unsaturated, straight or branched chain with 7 to 20 carbon atoms; acyl includes acetyl, pivaloyl, substituted or unsubstituted benzoyl and benzyloxycarbonyl; aralkyl includes benzyl.

The compounds of formula I of this invention are mercaptoacylamino acids and S-substituted mercaptoacylamino acids. Mercaptoacylamino acids have an inhibitory activity against angiotensin converting enzyme and therefore they are useful as antihypertensive agents. S-substituted mercaptoacylamino acids release mercaptoacylamino acids by enzymatic and/or chemical cleavage when administered to men or animals.

The compounds of formula I can be produced by the following methods.

An acid of the formula II



(II)

is reacted with a functional derivative of alkanoic acid of the formula III

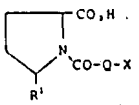
$$R^3S—Q—CO_2H \qquad (III)$$

preferably by one of the known procedures wherein the compound III is activated by forming a mixed anhydride, a symmetrical anhydride, acid chloride or an active ester prior to reaction with the acid II to produce the compounds of the formula I. The resulting compound can then be converted to the compound of formula I, wherein R^2 is hydrogen, by hydrolysis or reduction (such as by acid treatment with hydrochloric acid or p-toluenesulfonic acid for example; by alkali treatment, with sodium hydroxide or ammonia, for example; by catalytic reduction, with palladium-carbon, for example; or by alkaline metal treatment in liquid ammonia).

In another way, the compounds of formula I are produced by reacting an acid of formula II with a functional derivative of a haloalkanoic acid of the formula IV

$$X—Q—CO_2H \qquad (IV)$$

and by reacting the resulting haloacid of the formula V



(V)

with thiobenzoic acid. The resulting compound can then be converted to the compound of formula I, wherein R^2 is hydrogen, by hydrolysis or reduction in the same manner as above.

In the formulae (III, IV and V)

R^3 is benzoyl,

X is halogen (e.g. bromine or chlorine).

The compounds of formula I synthesized by the above methods can be in the form of conventional salts generally used in medicine, e.g. the sodium, potassium, calcium, aluminium, ammonium, diethylamine or triethanolamine salt.

The compounds of formula I have stereoisomers which are within the scope of this invention because they have asymmetric carbon atoms.

Examples are shown below, although this invention is not limited to these examples.

Example 1

1-[(2S)-S-Benzoyl-3-mercapto-2-methylpropanoyl]-5-phenyl-2-pyrrolidinecarboxylic Acid

22.8 g of 5-phenyl-2-pyrrolidinecarboxylic acid hydrochloride and 41.7 ml of triethylamine are dissolved in 700 ml of anhydrous acetone. To this solution, 24.3 g of (2S)-S-benzoyl-3-mercapto-2-methylpropanoyl chloride is added dropwise with stirring under ice-cooling. After the addition, the mixture is stirred under ice-cooling for 1 hour and at room temperature for another 1 hour. To the mixture, 5.7 ml of acetic acid is added, and the precipitate is removed by filtration. The filtrate is concentrated in vacuo and the produced oil is dissolved in 500 ml of ethyl acetate. The organic layer is washed with water and saturated sodium chloride solution, dried over magnesium sulfate, and concentrated in vacuo to give 53.8 g of oil. The oil is purified by silica gel column chromatography to give the titled compound, yield 30.2 g (76%), $[\alpha]_D^{29}$—45.6° (c=1.0, methanol).

IR ($CHCl_3$, cm^{-1}) 1725, 1650, 1580, 910.

Example 2

1-[(2S)-3-Mercapto-2-methylpropanoyl]-5-phenyl-2-pyrrolidinecarboxylic Acid

20 g of 1-[(2S)-S-benzoyl-3-mercapto-2-methylpropanoyl]-5-phenyl-2-pyrrolidinecarboxylic acid is dissolved in 50 ml of methanol. To this solution 100 ml of conc. ammonia is added, and the mixture is stirred at room temperature for 1.5 hours. Excess ammonia and methanol are removed in vacuo, and the residue is washed with ethyl acetate. The aqueous layer is acidified with conc. hydrochloric acid, and extracted with ethyl acetate. The organic layer is washed with saturated sodium chloride solution, dried over magnesium sulfate, and concentrated in vacuo to give the titled compound, yield 8.5 g (58%), mp. 138.0—142.5°C (ethyl acetate), $[\alpha]_D^{29}$—29.4° (c=1.0, methanol).

IR (nujol, cm^{-1}, to be applied hereinafter unless specified) 2650, 1740, 1600, 750, 705.

Example 3

α- and β-1-(S-Benzoyl-3-mercaptopropanoyl)-5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic Acid

12.2 g of 5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic acid hydrochloride and 9.6 g of sodium carbonate are dissolved in 200 ml of water. To this solution, 11.4 g of S-benzoyl-3-mercaptopropanoyl chloride is added dropwise with stirring under ice-cooling. After the addition, the mixture is stirred under ice-cooling for 1 hour and at room temperature for another 1 hour. This reaction solution is washed with ethyl acetate, acidified with conc. hydrochloric acid, and extracted with ethyl acetate. The organic layer is washed with saturated sodium chloride solution, dried over magnesium sulfate, and concentrated in vacuo to give crystals (α-acid). The crystals are collected on a filter, yield 9.4 g (47%). The filtrate is concentrated in vacuo, and purified by silica gel column chromatography to give β-acid, yield 2.4 g (12%).

	α-acid	*β-acid*
	mp. 210—211°C (ethyl acetate-methanol)	—
	IR 3200, 1742, 1722, 1655, 1615, 1595, 1450, 1236, 1204, 912	1740, 1658, 1625, 1240, 1209, 915 (neat, cm^{-1})
	TLC: Rf value[a] 0.28	0.38

(a) silica gel, benzene-ethyl acetate-acetic acid (25:25:1)

Example 4

α- and β-5-(2-Hydroxyphenyl)-1-(3-mercaptopropanyl)-2-pyrrolidinecarboxylic Acid

i) 4.0 g of α-1-(S-benzoyl-3-mercaptopropanoyl)-5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic acid obtained in Example 3 is dissolved in 50 ml of methanol. To this solution, 50 ml of conc. ammonia is added, and the mixture is stirred at room temperature for 1.5 hours. Excess ammonia and methanol is removed in vacuo, and the residue is washed with ethyl acetate. The aqueous layer is acidified with conc. hydrochloric acid to give α-acid, yield 2.5 g (85%).

ii) 2.0 g of β-1-(S-benzoyl-3-mercaptopropanoyl)-5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic acid obtained in Example 3 is dissolved in 20 ml of methanol. To this solution, 20 ml of conc. ammonia is added, and the mixture is treated in the same manner as (i) to give β-acid, yield 1.2 g (82%).

	α-acid	*β-acid*
	mp. 213—214°C (ethyl acetate-benzene)	209—210°C (ethyl acetate-benzene)
	IR 3160, 1720, 1618, 1598, 1450	3180, 1718, 1620, 1600, 1450
	TLC: Rf value[b] 0.38	0.38

(b) silica gel, ethyl acetate-ethanol-acetic acid (40:1:1).

Example 5

α- and β-1-(S-Benzoyl-3-mercaptopropanoyl)-5-(4-hydroxyphenyl)-2-pyrrolidinecarboxylic Acid

To the mixture of 12.2 g of 5-(4-hydroxyphenyl)-2-pyrrolidinecarboxylic acid hydrochloride, 9.6 g of sodium carbonate, 250 ml of water and 100 ml of ether, 11.4 g of S-benzoyl-3-mercaptopropanoyl chloride is added dropwise with stirring under ice-cooling. After the addition, the mixture is stirred under ice-cooling for 1 hour and at room temperature for another 1 hour. This reaction solution is acidified with conc. hydrochloric acid, and extracted with ethyl acetate. The organic layer is washed with saturated sodium chloride solution, dried over magnesium sulfate, and concentrated in vacuo to give crystals (α-acid). The crystals are collected on a filter, yield 6.2 g (31%). The filtrate is concentrated in vacuo, and purified by silica gel column chromatography to give β-acid, yield 3.6 g (18%).

	α-acid	*β-acid*
	mp. 164—166°C (ethyl acetate-methanol)	—
	IR 3170, 1714, 1654, 1611, 1206, 908	3340, 1750, 1653, 1207, 914 (neat, cm^{-1})
	TLC: Rf value[a] 0.22	0.32

(a) Conditions are the same as Example 3.

Example 6

α- and β-5-(4-Hydroxyphenyl)-1-(3-mercaptopropanoyl)-2-pyrrolidinecarboxylic Acid

i) 4.0 g of α-1-(S-benzoyl-3-mercaptopropanoyl)-5-(4-hydroxyphenyl)-2-pyrrolidinecarboxylic acid obtained in Example 5 is dissolved in 50 ml of methanol. To this solution, 50 ml of conc. ammonia is added, and the mixture is treated in the same manner as Example 2 to give α-acid, yield 2.2 g (74%).

ii) 2.0 g of β-1-(S-benzoyl-3-mercaptopropanoyl)-5-(4-hydroxyphenyl)-2-pyrrolidinecarboxylic acid obtained in Example 5 is dissolved in 20 ml of methanol. To this solution, 20 ml. of conc. ammonia is added, and the mixture is treated in the same manner as Example 2 to give β-acid, yield 0.8 g (54%).

	α-acid	*β-acid*
	mp. 154—157°C (ethyl acetate-benzene)	—
	IR 3300, 1746, 1594, 1239, 1188	3240, 1710, 1610, 1210 (neat, cm^{-1})
	TLC: Rf value[a] 0.21	0.19

(a) Conditions are the same as Example 3.

Example 7

α- and β-1-[(2S)-S-Benzoyl-3-mercapto-2-methylpropanoyl]-5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic Acid

12.2 g of 5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic acid hydrochloride and 9.6 g of sodium carbonate are dissolved in 200 ml of water. To this solution, 12.1 g of (2S)-S-benzoyl-3-mercapto-2-methylpropanoyl chloride is added dropwise with stirring under ice-cooling. After the addition, the mixture is stirred under ice-cooling for 1 hour and at room temperature for another 1 hour. This reaction solution is washed with ethyl acetate, acidified with conc. hydrochloric acid, and extracted with ethyl acetate. The organic layer is washed with saturated sodium chloride solution, dried over magnesium sulfate, and concentrated in vacuo to give crystals (α-acid). The crystals are collected on a filter, yield 9.2 g (44%). The filtrate is concentrated in vacuo, purified by silica gel column chromatography to give β-acid, yield 2.6 g (13%).

	α-acid	*β-acid*
	mp. 204—205°C (ethyl acetate-benzene)	—
	$[\alpha]_D^{25}$ −17.5° (c=1.0, methanol)	−41.6° (c=1.0, methanol)
	IR 3310, 1713, 1668, 1621, 1598, 1446, 1209, 907	1739, 1655, 1620, 1600, 1450, 1208, 915 (neat, cm^{-1})
	TLC: Rf value[b]	
	0.60	0.68

(b) Conditions are the same as Example 4.

Example 8

α- and β-5-(2-Hydroxyphenyl)-1-[(2S)-3-mercapto-2-methylpropanoyl]-2-pyrrolidinecarboxylic Acid

i) 4.1 g of α-1-[(2S)-S-benzoyl-3-mercapto-2-methylpropanoyl]-5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic acid obtained in Example 7 is dissolved in 50 ml of methanol. To this solution, 50 ml of conc. ammonia is added, and the mixture is treated in the same manner as Example 4 to give α-acid, yield 2.8 g (90%).

ii) 2.1 g of β-1-[(2S)-S-benzoyl-3-mercapto-2-methylpropanoyl]-5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic acid obtained in Example 7 is dissolved in 25 ml of methanol. To this solution, 25 ml of conc. ammonia is added, and the mixture is treated in the same manner as Example 4 to give β-acid, yield 1.1 g (71%).

	α-acid	*β-acid*
	mp. 241—242°C (ethyl acetate-methanol)	233—234°C (ethyl acetate-benzene)
	$[\alpha]_D^{25}$ −22.0° (c=1.0, methanol)	−56.8° (c=1.0, methanol)
	IR 3310, 1720, 1613, 1599, 1460	3320, 1723, 1616, 1600, 1462
	TLC: Rf value[b]	
	0.54	0.55

(b) Conditions are the same as Example 4.

Example 9

α_1-, β_1-, α_2-, and β_2-1-(S-Benzoyl-3-mercaptopropanoyl)-5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic Acid

35.8 g of (±)-2-(2-hydroxyphenyl)-5-pyrrolinecarboxylic acid is reacted with 34.5 g of (−)-1,2-diphenylethylamine to give the diastereoisomer salt. The salt is fractionally recrystallized from ethanol to give (−)-2-(2-hydroxyphenyl)-5-pyrrolinecarboxylic acid (−)-1,2-diphenylethylamine salt, yield 20.0 g (57%), mp. 193—194°C, $[\alpha]_D^{28}$ −90.0° (c=1.0, methanol). The residual salt in the filtrate is fractionally recrystallized from chloroform to give (+)-2-(2-hydroxyphenyl)-5-pyrrolinecarboxylic acid (−)-1,2-diphenylethylamine salt, yield 18.0 g (51%), mp. 138—140°C, $[\alpha]_D^{28}$ −20.3° (c=1.2, methanol).

Each salt is treated with sodium hydroxide to give sodium (−)-2-(2-hydroxyphenyl)-5-pyrrolinecarboxylate, yield 10.6 g, mp. 250°C and over (dec.), $[\alpha]_D^{27}$ −249.5° (c=0.6, water), and sodium (+)-2-(2-hydroxyphenyl)-5-pyrrolinecarboxylate, yield 7.9 g, mp. 250°C and over (dec.), $[\alpha]_D^{26}$ +249.4° (c=0.6, water).

i) 6.82 g of sodium (−)-2-(2-hydroxyphenyl)-5-pyrrolinecarboxylate is dissolved in 120 ml of 0.5 N hydrochloric acid, and hydrogenated with 300 mg of platinum oxide to give (−)-5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic acid hydrochloride.

All of thus obtained product is dissolved in the mixture of 10.62 g of triethylamine, 300 ml of acetone and 15 ml of water. To the reaction mixture, 6.9 g of S-benzoyl-3-mercaptopropanoyl chloride is added dropwise with stirring under ice-cooling. After the addition, the mixture is stirred at room temperature for 1 hour, and acidified with hydrochloric acid. Acetone is removed, and then the mixture is extracted with ethyl acetate. The organic layer is washed with saturated sodium chloride solution, dried over magnesium sulfate, and evaporated to give oil. The oil is separated and purified by column chromatography to give α_1-acid, yield 5.91 g.

Another fraction is reacted with dicyclohexylamine to give β_1-acid dicyclohexylamine salt, yield 0.48 g.

	α_1-acid	β_1-acid dicyclohexylamine salt
	mp. 89—92°C (dec.)	191—192°C
	$[\alpha]_d^{25}$+47.4° (c=1.0, methanol)	−11.2° (c=0.5, methanol)
	IR 3400, 1750, 1660, 1575, 1200, 1175, 905	3300, 1655, 1630, 1555, 1400, 1195, 910
	TLC: Rf value[a] 0.59	0.57

(a) silica gel, ethyl acetate-chloroform-acetic acid (7:5:1), to be applied hereinafter.

ii) 6.82 g of sodium (+)-2-(2-hydroxyphenyl)-5-pyrrolinecarboxylate is treated in the same manner as i) to give α_2-acid, yield 4.7 g, and β_2-acid dicyclohexylamine salt, yield 0.15 g.

	α_2-acid	β_2-acid dicyclohexylamine salt
	mp. 91—93°C (dec.)	192—193.5°C
	$[\alpha]_D^{25}$−49.8° (c=0.9, methanol)	+11.6° (c=0.5, methanol)
	IR 3400, 1750, 1660, 1575, 1200, 1175, 905	3300, 1655, 1630, 1555, 1400, 1195, 910
	TLC: Rf value[a] 0.59	0.57

Example 10

α_1- and α_2-5-(2-Hydroxyphenyl)-1-(3-mercaptopropanoyl)-2-pyrrolidinecarboxylic Acid

i) 1.0 g of α_1-1-(S-benzoyl-3-mercaptopropanoyl)-5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic acid is dissolved in 10 ml of conc. ammonia. The mixture is stirred at room temperature for 1 hour. Ammonia is removed, and then the mixture is washed with ethyl acetate. The aqueous layer is acidified with hydrochloric acid, and extracted with ethyl acetate. The organic layer is washed with saturated sodium chloride solution, dried over magnesium sulfate, and evaporated to give α_1-acid, yield 0.58 g.

ii) 1.0 g of α_2-1-(S-benzoyl-3-mercaptopropanoyl)-5-(2-hydroxyphenyl)- 2-pyrrolidinecarboxylic acid is treated in the same manner as i) to give α_2-acid, yield 0.58 g.

	α_1-acid	α_2-acid
	mp. 197—198°C (dec.) (ethyl acetate-cyclohexane)	198—199°C (dec.) (ethyl acetate-cyclohexane)
	$[\alpha]_D^{25}$+34.7° (c=0.5, methanol)	−35.3° (c=0.5, methanol)
	IR 3360, 1720, 1685, 1605, 1585, 1280, 1165, 760	3360, 1720, 1685, 1605, 1585, 1280, 1165, 760
	TLC: Rf value[a] 0.58	0.58

The potent antihypertensive effect of the compound (I) and related salts of this invention is clear in the comparison of the pharmacological activity between the known compound and the compounds of this invention as explained below. The inhibitor of angiotensin-converting enzyme which converts biologically inactive decapeptide, angiotensin I, to active octapeptide, angiotensin II, is found to be useful as antihypertensive medicine. In view of the above, we investigated the pharmacological activities of the compounds of the present invention from the aspect of inhibitory activity against the enzyme.

Pharmacological Test 1

As the method of measurement of angiotensin-converting enzyme activity, bioassay for the contractile response of isolated smooth muscle or the pressor response of normal animals and biochemical assay for the enzyme isolated from lung or other organs of animals are known. The former is found more advantageous than the latter for the examination of the convertion of angiotensin I to angiotensin II in vivo.

In the present study, therefore, we adopted the bioassay for contractile response of isolated guinea-pig ileum to angiotensin I.

Measurement of inhibitory activity of angiotensin-converting enzyme.

Isolated guinea-pig ileum was suspended in the organ bath containing 20 ml of Tyrode's solution of 30°C gassed with 95% O_2+5% CO_2. The contraction induced by the addition of angiotensin I (0.1 μg/ml) at intervals of 10 minutes was recorded on a recticorder (Nihon Koden) for 90 seconds using FD pick up (ST-1T-H, Nihon Koden).

The test compounds were added to the bath 5 minutes before the addition of angiotensin I.

The inhibitory activity of angiotensin-converting enzyme was calculated by the following formula.

$$\frac{A-B}{A}\times 100$$

A: contractile intensity of angiotensin I before addition of the compound
B: contractile intensity of angiotensin I after addition of the compound

From the fact that kininase II, which destroys bradykinin having contractile action of isolated guinea-pig ileum, is thought to be identical with angiotensin-converting enzyme, augmentation of the contractile response to bradykinin by test compounds was examined by using bradykinin (0.005 μg/ml) in place of angiotensin I according to the above mentioned method.

The results are shown in a table. All of the test compounds inhibited the contractile response to angiotensin I, while they enhanced the response to bradykinin.

Pharmacological Test 2

The activity of angiotensin-converting enzyme was measured by spectrophotometry according to the method of Biochem. Pharmacol., *20*, 1637 (1971). That is, the absorbance of hippuric acid was measured, which is liberated by incubating hippuryl-L-histidyl-L-leucine (HHL) as substrate in the presence of angiotensin-converting enzyme extracted from rabbit lung.

Measurement of Inhibitory Activity of Angiotensin-converting Enzyme

The reaction mixture is as follows:
100 mM phosphate buffer (pH 8.3)
300 mM sodium chloride
5 mM HHL
10^{-3}—10^{-9} M enzyme inhibitor
5 mU enzyme

0.25 ml of the above mixture was incubated at 37°C for 30 minutes and the reaction was stopped by adding 0.25 ml of 1 N hydrochloric acid. To this solution, 1.5 ml of ethyl acetate was added in order to extract hippuric acid. 1.0 ml of ethyl acetate layer was collected and evaporated to dryness, and the residue obtained was dissolved in 1.0 ml of water. The absorbance of this solution was measured at 228 nm.

The inhibitor activity of angiotensin-converting enzyme was calculated by the following formula:

$$\text{Percent inhibition}=\frac{A-B}{A}\times 100$$

A: absorbance of reaction solution before addition of the compound
B: absorbance of reaction solution after addition of the compound

Concentration of Compound Producing 50% Inhibition of Angiotensin-converting Enzyme (IC_{50})

The solution containing compounds at the concentration of 1×10^{-3} M to 1×10^{-9} M was incubated and percent inhibition at each concentration was calculated according to the above formula, and then IC_{50}, concentration of compound producing 50% inhibition of the enzyme activity, was determined.

The results are shown in a table.

The compounds examined in these tests are shown below. The compounds of this invention

Compound A: α-5-(2-hydroxyphenyl)-1-(3-mercaptopropanoyl)-2-pyrrolidinecarboxylic acid
Compound B: α-5-(4-hydroxyphenyl)-1-(3-mercaptopropanoyl)-2-pyrrolidinecarboxylic acid
Compound C: α-5-(2-hydroxyphenyl)-1-[(2S)-3-mercapto-2-methylpropanoyl]-2-pyrrolidinecarboxylic acid
Compound D: β-5-(2-hydroxyphenyl)-1-[(2S)-3-mercapto-2-methylpropanoyl]-2-pyrrolidinecarboxylic acid
Compound E: α_1-5-(2-hydroxyphenyl)-1-(3-mercaptopropanoyl)-2-pyrrolidinecarboxylic acid
Known compound
Compound Z: (4R)-3-[(2S)-3-mercapto-2-methylpropanoyl]-4-thiazolidinecarboxylic acid

Table

Inhibitor Activity Against Angiotensin-converting Enzyme

Compound	*Angiotensin I* IC_{50}*(M)	*Bradykinin* AC_{50}**(M)	*Angiotensin-converting enzyme* IC_{50}***(M)
A	1.7×10^{-7}	1.8×10^{-9}	5.6×10^{-7}
B	4.8×10^{-7}	9.8×10^{-9}	4.4×10^{-7}
C	1.6×10^{-7}	2.0×10^{-9}	1.1×10^{-6}
D	1.5×10^{-7}	2.6×10^{-9}	1.2×10^{-6}
E	7.6×10^{-8}	9.5×10^{-10}	6.5×10^{-8}
Z	1.7×10^{-7}	2.6×10^{-9}	2.6×10^{-7}

*Concentration of compound producing 50% inhibition of the contractive action of angiotensin I on the guinea-pig ileum.

**Concentration of compound producing 50% augmentation of the contractive action of bradykinin on the guinea-pig ileum.

***Concentration of compound producing 50% inhibition against angiotensin-converting enzyme.

Toxicity Test

Acute toxicity of compound A is low, that is the LD_{50} value is 1000—1250 mg/kg.

(Experimental animals)

The male ddy-std strain mice (4 weeks of age, weighing 19—21 g) were placed in a breeding room of constant temperature and humidity (23±1°C, 55±5%) and fed freely pellet diet (CE-2, Clea Japan Inc.) and water ad. libitum for a week. The mice showing the normal growth were selected for the experiment.

(Method of Administration)

Test compounds are suspended in 0.5% tragacanth solution, and administered intraperitoneally in a dose of 0.5 ml of 20 g body weight.

It is found from the above pharmacological tests that the compounds I of this invention are useful as antihypertensive agents. The compounds can be given with the combination of diuretics as other antihypertensive agents can generally at present. The dosage forms are tablet, capsule, granule, powder, suppository, injection etc. In the treatment of hypertension, these preparations can contain not only general excipients but also other antihypertensive agents such as reserpine, α-methyldopa, guanethidine, clonidine, hydralazine, etc. The dose is adjusted depending on symptoms, dosage form, etc. But, usual daily dosage is 1 to 5000 mg, preferably 10 to 1000 mg, in one or a few divided doses.

The following are examples of formulation.

(1) Oral Drug

a. tablet

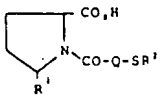
compound A	30 mg
lactose	150 mg
crystalline cellulose	50 mg
calcium carboxymethylcellulose	7 mg
magnesium stearate	3 mg
Total	240 mg

compound A	150 mg
lactose	60 mg
crystalline cellulose	30 mg
calcium carboxymethylcellulose	7 mg
magnesium stearate	3 mg
Total	250 mg

compound E	50 mg

Claims

1. A compound of the formula I



(I)

wherein

R^1 is phenyl, hydroxyphenyl, mercapto-lower alkyl, acylmercapto-lower alkyl, higher alkyl, higher alkenyl, cycloalkyl, aralkyl, aralkenyl, furyl, thienyl, imidazolyl, pyridyl, naphthyl, benzofuryl,

benzothienyl indolyl, substituted higher alkyl, substituted higher alkenyl, substituted cycloalkyl, substituted aralkyl, substituted aralkenyl, substituted phenyl, substituted furyl, substituted thienyl, substituted imidazolyl, substituted pyridyl, substituted naphthyl, substituted benzofuryl, substituted benzothienyl, or substituted indolyl wherein the substituent(s) is or are lower alkyl, hydroxy-lower alkyl, hydroxy, mercapto, lower alkoxy, lower alkylenedioxy, acyloxy, acylmercapto, halogen, nitro, amino, lower alkylamino, acylamino or carboxy, except hydroxyphenyl;

R^2 is hydrogen or benzoyl, and

Q is a straight or branched alkylene with 1 to 3 carbon atoms;

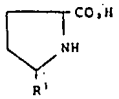
or a salt thereof.

2. A compound as claimed in claim 1, wherein R^1 is phenyl, 2-hydroxyphenyl or 4-hydroxyphenyl.

3. A compound as claimed in claim 1 or 2, wherein Q is $—CH_2—$, $—CH_2CH_2—$ or $—CH(CH_3)CH_2—$.

4. A composition comprising a compound as claimed in claim 1 in an amount sufficient to reduce blood pressure and at least one pharmaceutically acceptable excipient(s).

5. Process for manufacturing a comound as claimed in claim 1, which comprises reacting an acid of the formula II



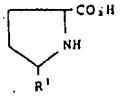
(II)

with a functional derivative of alkanoic acid of the formula III

$$R^3S—Q—CO_2H \qquad (III)$$

and optionally, subjecting the resulting compound to hydrolysis, reduction or ammonolysis, wherein R^1, R^2 and Q are as defined in claim 1, and R^3 is benzoyl.

6. Process for manufacturing a compound as claimed in claim 1, which comprises reacting an acid of the formula II

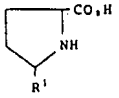


(II)

with a functional derivative of haloalkanoic acid of the formula IV

$$X—Q—CO_2H \qquad (IV)$$

to produce a compound of the formula V



(II)

and reacting the compound of the formula (V) with thiobenzoic acid, and optionally subjecting the resultant compound to hydrolysis or reduction, wherein R^1, R^2 and Q are as defined in claim 1, R^3 is benzoyl and X is halogen.

7. A compound as claimed in claim 1, substantially as hereinbefore described in any one of Examples 1 to 10.

8. A process for manufacturing a compound as claimed in claim 1, substantially as hereinbefore described in any one of Examples 1 to 10.

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